CSF ADENOSINE DEAMINASE (ADA) ACTIVITY IN PATIENTS WITH MENINGITIS

Dissertation submitted in partial fulfillment of the requirement for the award of the degree of

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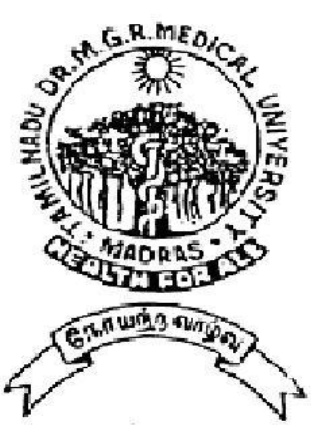

THE TAMILNADU Dr. M.G.R. MEDICAL UNIVERSITY,

CHENNAI, TAMILNADU.

CERTIFICATE

This is to certify that this thesis entitled "**CSF Adenosine Deaminase (ADA) Activity in patients with Meningitis**" is a bonafide work of **Dr. Sandhya. S,** for the degree of M.D. General Medicine under my guidance and supervision.

The method of work and the results embodied have been checked by me time to time and are contributory to the knowledge of the subject.

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DECLARATION

I, **Dr. Sandhya. S,** declare that, I carried out this work on, "**CSF ADENOSINE DEAMINASE (ADA) ACTIVITY IN PATIENTS WITH MENINGITIS**" at the Department of Medicine, Govt. Rajaji Hospital during the period of April 2012 to September 2012. I also declare that this bonafide work or a part of this work was not submitted by me or any others for any award, degree, diploma to any other University, Board either in India or abroad.

This is submitted to The Tamilnadu Dr. M. G. R. Medical University, Chennai in partial fulfillment of the rules and regulations for the M.D degree examination in General Medicine.

Place : Madurai **Dr. Sandhya. S**

Date :

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CONTENTS

INTRODUCTION

Meningitis is inflammation of the meninges (pia, arachnoid and duramater) covering the brain and the spinal cord. The most common cause of meningitis is infections due to viruses, bacteria, mycobacteria, fungi and other microorganisms. The Non-infectious causes include malignancy, chemical compounds, drugs and inflammatory conditions like CNS Sarcoidosis, SLE, Behcet's syndrome, etc. Meningitis can also be classified according to the temporal profile as acute, sub acute and chronic types.

Meningitis is a medical emergency as it can be life-threatening because of the inflammation's proximity to brain and spinal cord. If not recognised earlier, it can lead to serious long term neurological sequelae like deafness, epilepsy, hydrocephalus and cognitive deficits.

Lumbar puncture and CSF analysis is used to diagnose or exclude meningitis. Management involves prompt administration of antibiotics, and in appropriate situations, antiviral and anti-tuberculous agents according to the organism suspected. Steroids are

used as an adjunctive agent to prevent the complications from overactive inflammation.

Tuberculous meningitis (TBM) is an endemic disease in developing countries with an incidence of 7-21% [19]. It presents with gradual onset of symptoms and results in irreversible neurological complications and death if there is delay in diagnosis and start of effective treatment. But the available methods of diagnosis of TBM have too low sensitivity and specificity. Detection of acid fast bacilli (AFB) by light microscopy of the CSF smear is a rapid and specific method, but with a detection rate of only 30-40% [12]. Sensitivity of mycobacterial culture on Lowenstein- Jensen (L-J) medium is higher than microscopy but it needs several weeks of incubation. A number of genotypic assays based on nucleic acid amplification have been designed [13]. However, high costs involved in these tests preclude their use especially in developing countries.

In these situations, Adenosine Deaminase (ADA) estimation has been profoundly useful. ADA is an enzyme in the purine salvage pathway which is found in abundance in active T-lymphocytes. It is released by T cells during cell mediated immune response to the

tubercle bacilli. ADA levels have been considered by several researchers to differentiate tuberculous and nontuberculous diseases [6, 9, 10, and 14]. Its role in differentiating the tuberculous pleural, pericardial effusion and ascites from other causes has been well established. Several studies have also emphasized its importance as a simple, rapid, cost effective and fairly specific test in distinguishing TB meningitis from other causes of meningitis. Hence an attempt was made to estimate the CSF ADA level in patients with suspected meningitis and throw light on its use in differentiating the various types of meningitis.

REVIEW OF LITERATURE

Central nervous system infections represent a continuing diagnostic and therapeutic problem in our country and frequently present as medical emergencies [3].

When the infection predominantly involves the meninges and the subarachnoid space, it is known as meningitis. It presents with a characteristic combination of pyrexia, headache and meningism. Meningism consists of headache, photophobia and stiffness of the neck, often accompanied by other signs of meningeal irritation.

Signs of Meningeal Irritation:

Nuchal (cervical) rigidity is the most widely recognised and frequently encountered sign of meningeal irritation, and the diagnosis of meningitis is rarely made in its absence [1]. It is characterised by stiffness and spasm of the neck muscles, with pain on attempted voluntary movement as well as resistance to passive movement. Nuchal rigidity primarily affects the extensor muscles, and the most prominent early finding in meningeal irritation is resistance to passive flexion. The physician is unable to place the patient's chin on his chest, but the neck can be hyperextended;

rotatory and lateral movements may also be preserved. With more severe nuchal rigidity, retraction of the neck into a position of opisthotonus may occur. Rigidity may be absent in fulminating or terminal illness, when the patient is in coma, or in infants.

Kernig's and Brudzinski's neck sign are other meningeal signs. To elicit Kernig's sign, the more common method is to flex the hip and knee to right angles and then attempt to passively extend the knee [1]. This movement produces pain, resistance, and inability to fully extend the knee. Kernig's sign is positive in meningitis due to diffuse inflammation of the nerve roots and meninges. Brudzinski's neck sign is as follows- placing one hand under the patient's head and flexing the neck while holding down the chest with other hand causes flexion of hips and knees bilaterally [1].

These meningeal signs depend on the activation of protective reflexes that shorten the spine and immobilise it thus reducing the stretch on inflamed spinal structures. Resistance to forward flexion of neck (Brudzinski's sign) and extension of the legs (Kernig's sign) involve manoeuvres that oppose these protective flexor reflexes [2].

To avoid spinal flexion, the patient with meningitis may sit in bed with the hands placed far behind, the head thrown back, the hips and knees flexed and the back arched (Amoss's, Hoyne's or tripod sign)[1].

The severity of clinical features in meningitis varies according to the causative organism and CSF abnormalities help in distinguishing the cause of meningitis. Key goal of early management is to find out the causative organism and start a proper therapeutic regimen.

Causes of Meningitis:

It can be broadly classified as infective and non-infective causes.

Infective:

Bacteria:

Neonate	-	Gram-negative bacilli (Escherichia coli, Proteus), Group B Streptococci.
Preschool child	-	Haemophilus influenzae, Neisseria meningitidis.
Older child/ Adult	-	Streptococcus pneumoniae, Neisseria meningitidis, Listeria monocytogenes.

Viruses**:**

Enteroviruses (Echo, coxsackie, polio), Mumps, Influenza, Herpes Simplex, Varicella Zoster, Ebstein- Barr virus, HIV, Lymphocytic choriomeningitis, Mollaret's meningitis (herpes simplex virus type 2).

Protozoa and parasites : Toxoplasma, Amoeba, Cysticercus.

Fungi : Cryptococcus neoformans, Candida, Histoplasma, Blastomyces, Coccidiodes, Sporothrix.

Non-Infective:

Malignant disease: Breast cancer, Bronchial cancer, Leukaemia,Lymphoma.

Inflammatory disease: Sarcoidosis, SLE, Behcet's disease.

Most of the bacterial and viral infections cause acute meningitis whereas most mycotic infections, tuberculosis, syphilis, lyme disease, HIV infection and presumed non-infectious causes such as lymphoma, Sarcoidosis, Wegener's granulomatosis induce an inflammation of the leptomeninges of lesser intensity and more chronicity[2].

BACTERIAL MENINGITIS:

Bacterial meningitis may be defined as bacterial invasion and subsequent inflammation of pia-arachnoid mater and exudation in cerebrospinal fluid (CSF) of the subarachnoid space [3]. An organism entering this subarachnoid space can reach almost any part of brain and hence meningitis is almost always a cerebrospinal infection [2].

Epidemiology/Etiology:

About 4-6 cases per 1, 00,000 adults worldwide are infected every year [3]. Epidemiological data from India and other developing countries are lacking but the incidence is expected to be high.

The organisms most often responsible for community-acquired bacterial meningitis are Streptococcus pneumoniae, Neisseria meningitides, group B streptococci, Listeria monocytogenes and Haemophilus influenzae type b [4]. In developed nations, following the introduction of effective vaccination, the frequency of meningitis caused by H.influenzae has declined by 82 percent [3]. At present, pneumococcal and meningococcal meningeal infections predominate, especially in males.

Pneumococcal meningitis occurs in the very young and in older adults. Pneumococcal lung and sinus infections predispose the patients to meningeal infection.

Meningitis due to N.meningitidis has decreased with routine immunisation of 11 to 18 year olds with the tetravalent (serogroups A, C, W-135, and Y) meningococcal glycoconjugate vaccine. Presence of petechial or purpuric skin lesions provides an important diagnostic clue. Individuals with complement deficiencies are highly susceptible to meningococcal infections.

Enteric gram-negative bacilli cause meningitis in persons with diabetes, cirrhosis, alcoholism and in those with chronic UTI. Gram negative meningitis can also complicate neurosurgical procedures.

Listeria meningitis is associated with exceptionally high mortality. It causes meningitis in neonates, pregnant women and immunocompromised individuals.

Staphylococcus aureus and coagulase negative staphylococci are important causes of meningitis, that occurs following invasive neurosurgical procedures, particularly shunting procedures for hydrocephalus [2].

Pathogenesis:

The common infections causing meningitis are transmitted via air-borne route. Bacteria after colonising the nasopharyngeal mucosa secrete IgA protease enzymes that breakdown the protective mucous barrier allowing bacterial attachment to the epithelium. The outer adhesive bacterial pili also play an important role in the attachment of the encapsulated bacteria to the nasopharyngeal mucosa. After attachment, bacteria invade the adjacent intravascular space and gain access to the blood stream and finally enter the CSF through the choroid plexus of the lateral ventricles, and other points where blood brain barrier is weak. Once bacteria reach the CSF, they rapidly multiply because host humoral mechanisms in CSF are deficient [3].

Alternatively, bacterial meningitis can occur by haematogenous spread, through congenital neuroectodermal defects, craniotomy sites, middle ear diseases, paranasal sinus infections and skull fractures.

Meningitis associated brain injury and neuronal death is not mediated simply by the presence of viable bacteria but occurs as a consequence of the host inflammatory reaction to bacterial

components. Host inflammatory mediators include complement, tumour necrosis factor, interleukin-1, interleukin-6 and C-reactive protein.

Stages in the pathogenesis of community- acquired bacterial meningitis:

1. Nasopharyngeal colonisation
2. Nasopharyngeal epithelial cell invasion
3. Invasion of meningeal vessels
4. Intravascular multiplication and bacteremia
5. Crossing of blood brain barrier; bacterial entry into CSF
6. Survival and multiplication in CSF

Clinical Presentation:

The characteristic symptoms and signs of bacterial meningitis are headache, fever, nuchal rigidity, photophobia, vomiting and lethargy or an altered level of consciousness. Other signs of meningeal irritation (Kernig's and Brudzinski's signs) are also present in majority of the patients. Seizures occur in approximately 23-40% of patients, typically in the first week of illness [3]. Focal

neurological signs complicate bacterial meningitis in 15-28% of patients and are caused by cortical vein thrombosis, cerebral artery spasm, subdural empyema or rarely brain abscess [3].

Vestibulo-cochlear nerve is commonly affected in this type of meningitis resulting in sensorineural deafness, either unilateral or bilateral. It can appear early or late in the course of the disease and is usually permanent. Involvement of the 6^{th} and 7^{th} cranial nerves can occur at any time during the disease process and is probably never persistent. The presence of bilateral 6^{th} nerve palsies, suggest raised intracranial pressure. Papilloedema is rare; its presence usually suggests another diagnosis such as space-occupying lesion.

Other signs indicative of elevated intracranial pressure and cerebral herniation are coma, unilateral or bilateral fixed and dilated pupils, decorticate or decerebrate posture, abnormal respiration (cheynes-stokes breathing, hyperventilation, apnoea or respiratory arrest) and loss of oculo-cephalic reflex. These signs are often of ominous prognostic significance.

Diagnosis:

Blood cultures should be immediately obtained when there is suspicion of bacterial meningitis and empirical antibiotics and adjunctive dexamethasone therapy started without delay [4]. If bacterial meningitis is suspected, lumbar puncture is an essential procedure. Prior to LP, CT/ MRI of the brain is necessary in patients in whom raised intracranial pressure or space- occupying lesion is suspected due to the presence of seizures, altered consciousness or papilloedema.

CSF Abnormalities in Bacterial Meningitis:

CSF changes are characteristic of bacterial meningitis. Opening CSF pressure is often elevated usually in the range of 200-500mm H_2O. The CSF appearance may be turbid due to the presence of neutrophils. The WBC count in CSF is high, about 100 – 10,000 cells/μL. Bacterial meningitis usually leads to a neutrophil predominance in CSF. The CSF glucose concentration is less than 40mg/dl and the CSF to serum glucose ratio <0.4. The CSF protein concentration is increased >45mg/dl. Gram's staining of CSF sediment is extremely important as it permits rapid and accurate

identification of the microorganism. The CSF culture is positive in approximately 70-85% of patients [3]. The probability of CSF culture being negative is increased if patient has received prior antimicrobial therapy. Polymerase chain reaction (PCR) is especially useful in such situations. It helps by detecting the DNA of causative organisms. A rapid etiologic diagnosis is possible with the use of counter-current immunoelectrophoresis and such other serological techniques. These tests detect capsular antigen in CSF and are useful if CSF microscopy is negative, especially, if patient has already received antibiotics.

Measurements of chloride concentrations in the CSF are not very useful but they are usually found to be low, probably reflecting dehydration and low serum chloride levels [2]. In contrast, CSF lactate dehydrogenase (LDH), although infrequently measured, can be of diagnostic and prognostic value. A rise in total LDH activity is consistently observed in patients with bacterial meningitis, especially due to rise in fractions 4 and 5, which are derived from granulocytes. Fractions 1 and 2 of LDH, presumably derived from brain tissue, are only slightly elevated in bacterial meningitis but rise sharply in patients who develop neurologic sequelae or later die.

Imaging Studies:

Chest films are essential in patients with bacterial meningitis because they may disclose an area of pneumonitis or abscess [2]. Sinus and skull films may provide clues to the presence of cranial osteomyelitis, paranasal sinusitis or mastoiditis which are also better visualised on CT scans. The CT scan is particularly useful in detecting lesions that erode the skull or spine and provide a route for bacterial invasion, such as tumours or sinus wall defects, as well as in demonstrating a brain abscess or subdural empyema. MRI with gadolinium enhancement may display the meningeal enhancement and cortical reaction, and both types of imaging, with appropriate techniques will demonstrate venous occlusions and adjacent infarctions [2].

Other Laboratory Findings**:**

Blood cultures should be obtained because they are positive in 40 to 60 percent of patients with H.influenzae, pneumococcal and meningococcal meningitis [3]. The leukocyte count in blood is elevated, especially immature forms. Severe hyponatremia due to

inappropriate ADH secretion can complicate meningitis in later stages.

Treatment:

Antibiotic therapy should be started soon after diagnosis is suspected or confirmed with characteristic CSF findings, without waiting to determine the causative organism.

Empirical Antibiotic Therapy:

Choice of empirical antibiotic is determined on the basis of the patient's age and underlying health status. Combination of vancomycin and a third- generation cephalosporin (ceftriaxone or cefotaxime) is the preferred antibiotic regimen for empirical treatment [3]. Depending on the clinical scenario and suspected etiologic organism, drugs like acyclovir and doxycycline are added to the combination therapy [4]. In young infants and older adults, in whom Streptococcus agalactiae and Listeria monocytogenes are prevalent; treatment with broad spectrum cephalosporins is supplemented with ampicillin. Metronidazole is added to cover gram-negative anaerobes in patients with otitis, sinusitis, or mastoiditis. Ceftazidime, cefepime, or meropenem is administered in

neurosurgical patients, as they provide coverage against P.aeruginosa.

Pathogen Specific Therapy:

Meningococcal meningitis**:**

Penicillin G is the drug of choice for meningococcal meningitis caused by susceptible strains. Generally, high-dose intravenous penicillin-G should be administered every 4 to 6 hours for patients with invasive meningococcal disease. Acceptable alternatives are ampicillin, cefotaxime and ceftriaxone. Chloramphenicol is indicated for patients with anaphylactic allergic reactions to penicillin.

Pneumococcal meningitis**:**

As a result of increase in penicillin-resistant Streptococcus pneumoniae, adult patients with pneumococcal infection should receive ceftriaxone plus vancomycin unless organism is demonstrated to be cephalosporin-sensitive. Penicillin-sensitive species can be treated with continued cefotaxime, ceftriaxone, or chloramphenicol, or even switched to penicillin-G. For penicillin-

resistant species, vancomycin is continued and rifampin may be added.

Parenteral treatment is given for about 14 days. Patients with pneumococcal meningitis should have a repeat LP done after 24-36 h after the initiation of antibiotics to document sterilisation of CSF. If there is failure of sterilisation, then it should be considered as presumptive evidence of antibiotic resistance. Patients with penicillin and cephalosporin resistance not responding to intravenous vancomycin may benefit from addition of intraventricular vancomycin [4].

Listeria Meningitis**:**

Ampicillin is the drug of choice for Listeria infection. Cotrimoxazole is given in penicillin allergic patients.

Staphylococcal Meningitis**:**

Nafcillin is the drug of choice in meningitis due to susceptible strains of S.aureus or coagulase-negative staphylococci. Vancomycin is administered in patients allergic to penicillin and in MRSA infections.

Gram-Negative Bacillary Meningitis**:**

For Gram-negative bacilli a minimum treatment of three weeks is needed. Parenteral treatment with third generation cephalosporins is the treatment of choice.

Adjunctive Therapy:

Dexamethasone is an important part of adjunctive management of meningitis. Dexamethasone is administered 20 min before antibiotic therapy [4]. Steroids help to decrease the inflammation associated with the disease process by inhibiting the production of cytokines. In paediatric population, the efficacy of steroids in reducing the complications of meningitis like deafness, focal neurological deficits has been well established. Despite conflicting results from earlier studies of corticosteroids in adults, recent trails have persuasively demonstrated a reduction in mortality and improved overall outcome if dexamethasone (10 mg intravenously) was administered 15-20 min before the first dose of antibiotic and the same dose repeated every 6 h for 4 days. The improvement was largely in patients who were infected with pneumococcus.

Prognosis:

Untreated, bacterial meningitis is usually fatal. In treated H. Influenzae and meningococcal meningitis, the mortality rate is around 5 percent; in pneumococcal meningitis, the rate is higher (15 percent) [2]. A disproportionate number of deaths from meningitis occur in infants and in the aged. In adults, the presence of bacteremia, coma, seizures, and a variety of co-morbid conditions-diabetes mellitus, alcoholism, multiple myeloma, and head trauma-all worsen the prognosis. Death in meningitis may be attributed to the effects of overwhelming infection, with bacteremia, hypotension or brain swelling and cerebellar herniation especially during the first 48 hrs.

VIRAL MENINGITIS:

Viral meningitis is inflammation of the meninges due to a viral infection and in the majority of cases is a benign, self-limiting illness.

'Aseptic meningitis' is a non-specific term indicating any form of meningitis where the CSF is bacteriologically sterile, accompanied by a lymphocytic pleocytosis and normal sugar content

[3]. Majority of cases are caused by viruses but a few cases are bacterial (mycoplasma, Q fever, other rickettsial infections) [2]. Because aseptic meningitis is rarely fatal, the precise pathologic changes are uncertain but are presumably limited to the meninges

Etiology:

The most common viruses causing meningitis are the Enteroviruses (poliovirus, coxsackie viruses, and echoviruses), HSV type 2, HIV, and arboviruses. Others are the lymphocytic choriomeningitis virus (LCMV), Epstein-Barr virus (EBV), influenza, adenovirus and the mumps virus.

Epidemiology:

Viral meningitis is not a nationally reportable disease. However, its incidence has been estimated around 75,000 cases per year [4]. All age groups are susceptible, but it is more common in those below 30 years of age. The peak incidence of enteroviral infections is during the months of August and September [2]. This is true also of infections caused by arboviruses.

Clinical Features:

Usually the onset is acute and the temperature is elevated, from 38 to 40°C. Headache that is more severe than that associated with other febrile states is the most frequent symptom. A variable degree of lethargy, irritability, and drowsiness may occur. Confusion, stupor, coma, and seizures mark the case as encephalitis rather than meningitis. Photophobia and pain on movement of the eyes are common additional complaints. Stiffness of the neck and spine on forward bending suggests the presence of meningismus, but at first it may be so slight as to pass unnoticed. Kernig's and Brudzinski's signs are often absent in the presence of manifest viral meningitis [2]. Systemic symptoms and signs aside from fever are infrequent and depend mainly on the systemic effects of the virus.

Laboratory Diagnosis:

CSF Examination:

CSF examination shows a clear fluid with mildly raised pressure, moderately increased protein, normal sugar content and pleocytosis ranging from 50-500 cells/mm^3. Organisms are not seen on Gram's stain of CSF. Usually the pleocytosis is mononuclear

except in the first 48 h of illness, when more than half the cells may be neutrophils, especially with infections due to echovirus 9, West Nile virus or mumps [2, 3]. High CSF cell counts in thousands is seen in LCMV and mumps and CSF glucose may be mildly reduced in LCMV, mumps, HSV-2, and VZV.

Nucleic acid amplification (PCR):

In Enteroviral and Herpes simplex virus infections of the CNS, PCR has become the diagnostic procedure of choice and more sensitive than viral culture. The other viruses that can be identified with PCR are cytomegalovirus (CMV), Ebstein- Barr virus (EBV), VZV, and human herpes virus (HHV-6) [4].

Viral culture**:**

Viral culture for the diagnosis of viral meningitis is possible sometimes. Specific viruses may also be isolated from throat swabs, stool, blood and urine, apart from CSF.

Serology:

Demonstration of virus-specific IgM antibodies in serum also suggest acute infection and if such antibodies are found in the CSF, acute CNS infection may be presumed. Seroconversion, however

does not occur immediately and in most instances, the diagnosis is made retrospectively by measuring the viral antibody levels in the acute and convalescent phases.

A four-fold increase in specific antibody titres in acute and convalescent serum or CSF is considered to be positive and even IgM antibodies may persist for months in the CSF and serum [3]. CSF oligoclonal gamma globulin bands occur in association with a number of viral infections, the associated antibodies often directed against viral proteins [4].

Other laboratory studies**:**

Neuroimaging studies (MRI, CT) are not necessary in patients with uncomplicated viral meningitis, but should be performed in patients with altered sensorium, cranial nerve palsies or atypical CSF profile.

Specific Viral Etiologies:

Enteroviruses account for >85% of cases in which a specific etiology can be identified. These viruses cause meningitis in the summer and fall months, especially in children (< 15 years) [2, 4]. Familial outbreaks are usual. A number of echovirus and

coxsackievirus (particularly group A) infections are associated with exanthemata and may be associated with the greyish vesicular lesions of oral herpangina [2]. Pleurodynia, brachial neuritis, pericarditis, and orchitis are characteristic of some cases of group B coxsackievirus infections. Pain in the back and neck and in the muscles should suggest poliomyelitis. Lower motor weakness may occur with echo and coxsackievirus infections, but it is usually mild and transient in nature. Treatment is supportive and patients recover without sequelae.

Arboviral infections cause encephalitis rather than meningitis. Japanese encephalitis is arboviral encephalitis caused by a flavivirus. It is one of the most common causes of epidemic encephalitis in India. Children and young adults are mainly affected. A large number of subclinical cases occur. Onset is abrupt and patients present with rigors, seizures, involuntary movements like chorea, myoclonus and tics, and hyperthermia. The mortality rate ranges from 20%- 40%. The diagnosis can be confirmed if there is a fourfold increase of antibody titres between acute and convalescent sera or CSF. Viral culture is usually not successful.

Herpes Simplex Virus-2 meningitis is a major cause of meningitis in adults. About 25% of patients with acute genital herpes develop transient symptoms and signs of viral meningitis [3]. The virus may invade the sacral spinal cord resulting in bladder and bowel disturbances. Transverse myelitis is a rare complication of HSV-2 infection. HSV is thought to be the commonest cause of Mollaret's meningitis.

Varicella-Zoster Virus (VZV) causes Varicella in children and herpes zoster in adults and one of the neurological complications is meningoencephalitis. The other complications are self-limiting cerebellar ataxia, transverse myelitis and Reye's syndrome.

HIV meningitis may be associated with cauda equina neuritis. A mononucleosis type of infection (fever, rash, arthralgia, and lymphadenopathy) may occur at the time of seroconversion from recent HIV infection. This is associated with acute, self-limited aseptic meningitis. Aside from viral isolation and serologic tests and more recently, detection by polymerase chain reaction, few laboratory examinations are helpful.

Mumps meningitis occurs sporadically throughout the year, but the highest incidence is in late winter and spring [3]. Males are affected three times more frequently than females. Other manifestations of mumps infection- parotitis, orchitis, mastitis, oophoritis and pancreatitis- may be present. A definite past history of mumps aids in excluding the disease, as an attack confers lifelong immunity. Diagnosis is made by positive CSF viral culture or by detecting CSF IgM antibodies.

Treatment:

Treatment of almost all cases of viral meningitis is primarily symptomatic and includes the use of antipyretics, analgesics, and antiemetics. Monitoring of fluid and electrolyte status is must. Hospitalisation may not be required in immunocompetent patients with no alteration of consciousness, no focal signs or symptoms and classic CSF profile. Inpatient care is required only in patients with altered sensorium, seizures and focal deficits.

Acyclovir may be used in HSV-1 and 2, EBV and VZV infection and HAART in HIV meningitis. Seriously ill patients should receive intravenous acyclovir which can be followed by an

oral drug such as acyclovir, famciclovir, or valacyclovir for a total course of 7- 14 days.

An investigational drug, pleconaril, has been shown to be efficacious against infections caused by enterovirus [4].

Prognosis:

In adults there is full recovery following viral meningitis. In infants and neonates, the outcome is less certain. Learning disabilities, hearing loss have been reported in some studies.

TUBERCULOUS MENINGITIS:

Tuberculous meningitis is the commonest form of neurotuberculosis, accounting for 70-80% of cases [3]. Neurotuberculosis can affect the meninges, brain, or spinal cord either individually or in various combinations. Of these tuberculous meningitis presents as subacute or chronic meningitis.

Other organisms that can cause subacute meningitis include Cryptococcus neoformans, Histoplasma Capsulatum, Coccidiodes immitis and Trepenoma pallidum [4].

Chronic meningitis is most commonly diagnosed when a characteristic neurologic syndrome exists for >4 weeks and is associated with a persistent inflammatory response in the CSF [4].

In most western countries, the incidence of tuberculous meningitis, which parallels the frequency of systemic tuberculosis, has, until recently, decreased steadily. Since 1985 there has been a moderate increase in the incidence of systemic tuberculosis and tuberculous meningitis as a consequence of the HIV epidemic [2, 3]. In fact, tuberculosis may be the first clinical manifestation of HIV infection. In India also, the incidence of TBM reflects the incidence of tuberculosis in general, comprising about 10% of all cases of tuberculosis [3].

Etiology:

Tuberculosis of the nervous system is caused by the human strain of Mycobacterium tuberculosis. However, in immunocompromised persons, atypical mycobacteria, now called non-tuberculous mycobacteria (NTM) are important causes of infection. The principal organisms are Mycobacterium avium and Mycobacterium intracellulare.

Pathogenesis:

Tubercle bacilli hematogenously spread from the lungs during the stage of primary complex formation and settle in different areas of the CNS to form millet seed- sized (miliary) tubercles in the brain parenchyma [3]. These small subarachnoid or subependymal tubercles are called the Rich's foci. The highly vascular choroid plexus forms a common site for tubercle formation. Subsequent behaviour of each focus is individualised in the same person, one focus may progress while another just adjacent to it may remain static for years or regress. Some tubercles enlarge and begin to caseate. One or more of the tubercles may later rupture resulting in discharge of M. tuberculosis into the subarachnoid space. Since the choroid floats in the ventricular CSF where there is no supporting structure for the enlarging tubercle, this is the common site of rupture of a tubercle. Factors determining the development of meningitis include the number and virulence of the bacilli and the immune response of the host.

The immune response launched by the host is cellular rather than humoral. Macrophages ingest mycobacteria and destroy some

of them. The macrophages secrete cytokines, including TNF, which are thought to be responsible for the systemic manifestation of tuberculosis. The cell-mediated immunity (CMI) is usually beneficial to the host. However, the same immune response may give rise to the detrimental delayed type hypersensitivity (DTH) which results in host tissue damage in the CNS.

Pathology:

Meningeal Exudate**:**

The brunt of the pathologic process falls on the basal meninges, where a thick gelatinous exudate accumulates, obliterating the pontine and interpeducular cisterns and extending to the meninges around the medulla, the floor of the third ventricle and subthalamic region, the optic chiasm, and the undersurfaces of the temporal lobes. Cranial nerves are often involved by the inflammatory exudate as they traverse the subarachnoid space, indeed, far more often than with typical bacterial meningitis. Arteries may become inflamed and occluded, especially large vessels in the circle of Willis, and middle cerebral artery in the sylvian fissure, with infarction of brain. Histologically, the exudate

is serofibrinous. With treatment the exudate becomes fibrous, blocking the basal cisterns to cause hydrocephalus.

Meningoencephalitis**:**

Unlike the typical bacterial meningitides, the disease process is not confined to the subarachnoid space but frequently penetrates the pia and ependyma and invades the underlying brain, so that the process is truly a meningoencephalitis.

Hydrocephalus**:**

It is mostly of the communicating variety. Blockage of the basal cisterns in the interpeduncular fossa and the brainstem by exudate or granulation tissue is primarily responsible.

Vasculopathy**:**

The dense basal exudates, which surround large and small arteries including the lenticulostriate, cause a vasculopathy leading to occlusion of vessels. It results in focal and diffuse ischemic changes in the brain and also large infarcts. Recently, there is increasing identification of young persons with stroke, resulting solely from tubercular vasculitis, without associated meningitis. The belief is that the presence of tubercular antigen in the vessel wall

induces an immunologic phenomenon of vasculitis, which leads to infarction manifesting as stroke.

Tuberculoma Formation:

If a tubercle situated in the brain parenchyma starts progressing, it grows at the expense of the brain, which gets liquefied by the caseating process. The initial lesion is solitary or a cluster of microscopic foci, generally in the cortical or subcortical regions, consisting of a central area of necrosis surrounded by characteristic epitheloid and giant cell reaction. The site of tuberculoma is commonly the cerebellum in children and the cerebral hemispheres in adults. A fully formed tuberculoma has a creamy, white, gritty, caseating centre with crenated margins, surrounded by a firm to hard greyish rim of varying colour (pseudocapsule) of granulation tissue and compressed gliotic brain matter.

Spinal meningitis**:**

Spinal meningitis though often asymptomatic is usually present with cranial meningitis. Most cases are an extension of the tuberculous basal exudate downwards.

Clinical Features:

The classical form of the disease evolves through a prodromal stage, a stage of meningeal irritation, leading to a stage of diffuse or focal cerebral involvement. The onset is insidious, characterised by a vague illness lasting one or three weeks before meningeal symptoms and signs appear. Listlessness, apathy, irritability, lack of appetite, nausea, vomiting and abdominal pain are common features. Fever is of low grade or sometimes goes unnoticed. When meningeal irritation sets in, headache and vomiting becomes more prominent and neck rigidity and Kernig's sign are found.

In adults, the onset is frequently dominated by psycho behavioural manifestations. Focal or generalised epileptic fits may herald the onset in children. Abrupt onset of focal neurological deficit as the presenting feature is equally seen in both children and adults. Thus sudden onset of hemiparesis or monoparesis during the course of TBM is commonly recognised. The other modes of presentation of TBM include painful ophthalmoplegia, visual failure due to optochiasmatic arachnoiditis and other isolated cranial neuropathies.

As an assessment of severity of disease and a guide to prognosis, it is useful to stage patients clinically at the time of diagnosis, based on the British Medical Research Council classification.

Stage 1 (Early Disease)

Patient has meningeal signs only, consciousness is undisturbed, and no focal neurological signs are present.

Stage 2 (Medium severity)

Consciousness is disturbed but the patient is not comatose or delirious. Focal neurological signs and cranial nerve palsies may be present. These include hemiparesis, cranial neuropathies (II, III, IV, VI, VII, and VIII) and involuntary movements. Raised intracranial pressure may occur secondary to hydrocephalus.

Stage 3 (Advanced Disease)

The patient is deeply comatose with evidence of brainstem dysfunction, decerebrate or decorticate posturing, fixed dilated pupils, irregular pulse and respiration. Untreated patients progress through the three stages and usually die.

Cranial nerve palsies are seen in upto one-third of patients [3]. Papilloedema is common, usually without visual impairment. When visual impairment is noticed, the possibilities are optochiasmatic arachnoiditis, tuberculoma compressing the optic nerve or ethambutol toxicity. Oculomotor and abducens nerve palsies (III and IV) are common, whereas VII and VIII cranial nerve affection is less frequent. Rarely internuclear ophthalmoplegia and horizontal gaze paresis may be seen. This indicates intrinsic brainstem dysfunction and a poor prognosis, possibly because adjacent vital structures are involved. The finding of choroid tubercles on fundoscopy is very helpful in diagnosis.

The incidence of focal neurological deficit, monoplegia, hemiplegia and aphasia is reported to vary from 10-45 percent. Epileptic seizures may present at any stage. They are more frequent in children than adults and are usually the direct effect of meningeal inflammation on the cerebral cortex. When increasing drowsiness or seizures appear during treatment, it is mandatory to ascertain the exact cause, as cerebral edema, obstructive hydrocephalus, expanding intracranial tuberculoma and hyponatremia due to

inappropriate antidiuretic hormone (ADH) secretion must all be considered and managed appropriately.

Patients with TBM may have multiple intracranial tuberculomas. They may appear at a time when meningitis is resolving. In adults, supratentorial lesions are more frequent. Tuberculomas behave like a slow growing tumour. Located in cerebral hemisphere, it may cause focal seizures, progressive hemiparesis, speech disturbance and disturbance of cognitive function. A history of active tuberculosis elsewhere in the body or contact with an infective case is given by 25-30% of patients.

Spinal meningitis**:**

Tuberculous involvement of the spinal meninges may be either primary or secondary. Secondary spinal meningitis is commonly due to a spread of intracranial infection. It is reported in 10-30% of cases of TBM either during the course of its treatment or even years after the arrest of the disease. An uncommon source of secondary spinal meningitis is in association with tuberculous caries of the spine. Primary spinal meningitis consists of a rapid or slow, single or multiple level, ascending or transverse radiculomyelopathy. The

symptoms include fever, pain over the spine, root pains, paralysis, paraesthesiae, bladder disturbances and wasting of muscles. The disease may present in subacute or chronic forms. The clinical picture of the secondary spinal meningitis is initially dominated by the primary condition i.e. the cranial tuberculous meningitis. Symptoms and signs of spinal roots and cord (radiculomyelopathy) may supervene even while the intracranial lesion appears to be responding to therapy.

Investigations:

The key investigations include cerebrospinal fluid examination and neuroimaging.

Cerebrospinal Fluid Examination:

Cytology**:**

The typical CSF picture of TBM is clear fluid under raised pressure, with elevated levels of protein and cells, and low glucose levels. The clear or slightly turbid fluid may form a pellicle or cobweb clot on standing. Cobweb formation is not specific and should not be considered diagnostic. The cell count is raised (100-500 cells/mm^3). Characteristically, lymphocytes predominate in the

CSF. However, polymorphonuclear leucocytes may predominate early in the infection particularly during the first 10 days. This is transient, being replaced subsequently by a lymphocytic response. At times an initial mononuclear pleocytosis may change to a polymorphonuclear response upon initiation of treatment. This may be combined with a deteriorating clinical state, and it has been referred to as a therapeutic paradox [3]. The CSF protein is usually between 100-500 mg/dL. It may reach 1 gm/dL in patients with spinal block. The CSF glucose is less than 40 mg/dL in 50-85% of patients. An increase in CSF glucose with antituberculous therapy strengthens the diagnosis and indicates a better prognosis.

Microbiology**:**

Demonstration of tubercle bacilli in smear or culture is the only sure method of establishing the diagnosis of TBM. Most investigations reported from India achieve a positivity rate of only about 15-20% [3]. Detection rate may be increased by centrifuging 10- 20 ml of CSF for 30 minutes, preparing a thick smear from the deposit and examining it for up to 90 mins. It is believed that staining the cobweb may increase the probability of finding AFB.

Success with this traditional identification of tubercle bacilli in smears of CSF sediment stained by Ziehl-Neelson method is a function not only of their number but also of the persistence with which they are sought.

It takes several weeks (4 to 8 weeks) to obtain positive culture results in the conventional Lowenstein- Jensen (L-J) medium and the positivity is ~50% in adults[2, 3]. Culture remains the gold standard to make the diagnosis of TBM. Contemporary systems such as radiometric BACTEC and biphasic (Roche SeptiChek) culture improve the speed of mycobacterial recovery by 7- 10 days [2, 3]. But even with these methods, deferral of treatment to await results (2- 4 weeks in smear negative cases) is impractical. In order to overcome the difficulties encountered in isolating the organism, numerous tests have been devised over the last few years.

These tests can be broadly classified into two groups [2]:

Indirect tests which measure the host response to the organism:

These include the estimation of the adenosine deaminase level in CSF, the bromide partition test, and measurement of antibody to AFB in the CSF. Adenosine deaminase, an enzyme produced by T-

lymphocytes, is elevated in the CSF of 60-100% of patients with TBM [3, 7, 13, 19, and 37]. The bromide partition test measures the ratio of serum to CSF bromide after a loading dose. A ratio of less than 0.6 is said to be characteristic of TBM, but it is not routinely used as it is falsely positive in other forms of meningitis [3].

Serological tests are used to detect antibodies against mycobacterial antigens. Several antigens that have been evaluated include antigen 5, plasma membrane antigen, A60 antigen and glycolipids. These serological tests have been well studied in smear positive pulmonary tuberculosis, but the utility of these tests in TBM remains uncertain.

Direct tests which identify chemical components or antigens of the bacilli themselves**:**

These are the estimation of CSF tuberculostearic acid by gas chromatography or mass spectroscopy, the CSF 3-2'-ketohexyl indoline estimation, the identification of M. tuberculosis antigen in CSF by ELISA or RIA and the detection of M. tuberculosis DNA by PCR.

PCR:

PCR by DNA amplification permits the detection of small amounts of tubercle bacilli and now is widely available for clinical use. Some of the targets used in tuberculosis are IS6110 DNA sequence, 65KDa gene and MPB65 gene [3]. Nested PCR is a recently introduced technique whereby both sensitivity and specificity may be increased. PCR for M. tuberculosis is not affected by the presence of other infecting bacteria as may occur in an immunocompromised host. But the cost of PCR and sophisticated lab for the procedure precludes its use in developing countries.

Imaging**:**

Contrast CT scan and gadolinium enhanced MRI are very useful in the diagnosis of CNS tuberculosis and in assessing its complications. They are quite helpful in patients who develop raised intracranial pressure, hydrocephalus, or focal neurologic deficits. Tuberculomas can be visualised. MR or CT angiography may demonstrate vascular occlusive disease from granulomatous infiltration of the walls of arteries of the circle of Willis and their primary branches.

Hydrocephalus is the single most common abnormality and is reported in 50-80% of patients. The degree of hydrocephalus generally correlates with the duration of the disease. Enhancement of the basal meninges (60%), followed by cerebral infarction (28%), most frequently in the middle cerebral artery territory are other common findings.

In Spinal Meningitis, Myelography was the standard investigation for diagnosis as it yielded fairly specific images on radiography. Multiple filling defects, large areas of 'candle guttering', 'bundle of faggot sticks' appearance in the region of the cauda equina, arachnoid cysts, and blocked dye with a 'frayed' edge are some of the images vividly described. The T2 weighted MRI sequences of the spine show CSF loculation, obliteration of spinal subarachnoid space, loss of sharp outline of spinal cord in cervicothoracic region, clumping of nerve roots in lumbar region and nodular thick intradural enhancement after gadolinium injection.

CNS Tuberculosis and HIV infection:

Cognitive deficit is more commonly seen in HIV-positive patients. In these patients CT scan shows lesser meningeal

enhancement and communicating hydrocephalus than in those without HIV infection. Granulomatous exudate in the basal cisterns is less remarkable in the brain of HIV-infected patients. Periventricular and white matter infarcts are commonly seen. But changes of occlusive arteries in the basal cisternal vessels are uncommon.

Management of TBM:

The most important factor in preventing death and permanent neurological sequelae in patients with TBM is early initiation of treatment even if the diagnosis is not fully confirmed, as often happens.

Anti-Tuberculous Drugs**:**

Treatment is usually started with combination of Isoniazid (300mg/day), rifampicin (10mg/kg per day), pyrazinamide (30 mg/kg per day in divided doses), ethambutol (15-25 mg/kg per day in divided doses) and pyridoxine (50mg/day).

The primary goal of antituberculous chemotherapy is to kill tubercle bacilli rapidly, prevent the emergence of drug resistance and eliminate persistent bacilli from the host tissues to prevent relapse.

Isoniazid is the most powerful drug used in the treatment of tuberculosis, being responsible for killing the great majority of rapidly dividing bacilli. Because of its very good CSF penetration irrespective of meningeal inflammation, the efficacy of this drug is readily understandable.

Rifampicin's contribution to the treatment of TBM is relatively modest and significantly less than that in pulmonary disease.

Pyrazinamide has good oral absorption, excellent CSF penetration and sterilizing effect on the tubercle bacilli and this has resulted in mandatory inclusion of this drug in the intensive phase for the initial 2-3 months. Given the uncertain benefit and penetration of rifampicin, pyrazinamide should be given for the full duration of antitubercular therapy.

The total duration of treatment is not based on any firm control data but it has been customary to use long courses of treatment (18 months to two years) [3]. But studies have demonstrated that six months, nine months and one year regimens may be equally effective. An Indian study however considers short courses to be

inadequate and associated with increased relapse rate. Current UK guidelines recommend 12 months of ATT in uncomplicated cases of TBM extending to 18 months if pyrazinamide is omitted. Dexamethasone therapy is recommended for HIV-negative patients with tuberculous meningitis. The dose is 12–16 mg per day for 3 weeks, and then tapered over 3 weeks. Administration of steroids is found to be most beneficial when complications like raised intracranial pressure, cerebral oedema, stupor, focal neurological signs, spinal block, hydrocephalus and basal optochiasmatic arachnoiditis appear.

Surgical Intervention**:**

The most important role of surgery is the prompt reduction of obstructive hydrocephalus by a ventriculo-peritoneal shunt. In the case of tuberculoma, surgical excision is indicated when the anterior optic pathways are compressed with resultant visual impairment or for large tuberculomas with great rise of intracranial pressure not permitting time for effective medical therapy. In spinal meningitis, surgery has no role.

Prognosis:

The change in therapy over the years has not been reflected by improved prognosis. Despite therapeutic advances, the most important prognostic factors are still the stage of disease when the treatment is commenced, and the patient's age. Children under three years and adults over 65 years have a significantly worse prognosis. If treatment is started in stage 1 of TBM, almost all patients survive with little or no neurological deficit, whereas 50% of those first treated in stage 3 die, and those who recover may have permanent deficits. These sequelae are more common in children and include mental retardation, behavioural changes, seizures, visual or auditory loss, movement disorder, hemi/para/quadriplegia and various endocrinopathies due to hypothalamic- pituitary damage. Infection with HIV does not alter the prognosis of TBM, except that patients with CD4 count $<0.2 * 10^9$/L have a significantly reduced chance of survival. Failure to diagnose spinal meningitis and treat it early results in permanent disability.

Prevention:

The reported efficacy of BCG vaccine in preventing pulmonary tuberculosis varies from 0 to 80%. However its efficacy in preventing tuberculous meningitis ranges from 52 to 84 percent.

ADENOSINE DEAMINASE:

Adenosine deaminase (adenosine aminohydrolase, EC 3.5.4.4) or ADA is an enzyme involved in purine metabolism and it has been found in most mammalian tissues. A single genetic locus on chromosome 20 primarily controls ADA in man (Hirschhorn, 1975) [20]. Interest in this enzyme has been revived by the association between combined immunodeficiency and ADA deficiency (Meuwissen & Pollara, 1974) and further stimulated by the finding of a deficiency of the metabolically related enzyme nucleoside phosphorylase in a child with defective T-cell function (Giblett et al., 1975).

ADA catalyses the irreversible deamination of 2′deoxyadenosine and adenosine to deoxyinosine and inosine. Research of the 1980s increased the use of adenosine deaminase as a diagnostic tool in a variety of illnesses including meningitis and

tuberculosis. Two different isozymes have been identified- ADA1 and ADA2. ADA1 deficiency results in immunodeficiency and it is a Zn binding protein. ADA2 is produced by monocytes. The sequence of the enzyme is highly conserved from bacteria to humans. Adenosine deaminase has a $(beta/alpha)_8$ barrel structural motif, and contains a zinc atom in the catalytic pocket. Zinc or cobalt is necessary for catalytic activity.

ADA helps to detect or rule out Mycobacterium tuberculosis infection in pleural fluid in order to assist in the diagnosis of tuberculosis [14]; also to detect the infection in other body fluids such as peritoneal fluid or cerebrospinal fluid (CSF). ADA estimation in ascitic fluid at cut-off level of 30-32U/L has been found to be highly useful in diagnosis of tuberculosis [3]. In pleural fluid the cut –off is around 70 U/L [14]. In CSF, various cut-offs have been studied from 5-15U/L [7, 8, 15, 17, 21, 22]. ADA is mainly produced by immature T cells. ADA may be considered as an indicator of activation of cell mediated immunity. Its level is high in tuberculous pleural effusion, peritonitis and meningitis. A significant rise in levels of enzyme was observed during the first 10 days of therapy of

TBM, was followed by a gradual decline, and reached normal values after three to four months of treatment.

Moreover, estimation of ADA can be done by a simple method. Quantification is done spectrophotometrically as ADA catalyses the conversion of adenosine, resulting in liberation of ammonia, which produces a coloured indophenol compound [5]. Compared to other complex tests, the advantages of this test are

1. Feasibility of doing this test in simple labs by ordinary methods.
2. Early availability of results within 4-6 hrs.

Studies Demonstrating the Usefulness of CSF ADA in Differentiating Various Types of Meningitis:

Rajendra Prasad Et Al Conducted a Study in K.G's Medical College, Lucknow (U.P). In This Study Adenosine deaminase activity (ADA) was studied in cerebrospinal fluid of 29 cases of tuberculous meningitis, 15 cases of pyogenic meningitis, 12 cases of aseptic meningitis and 20 controls (patients without any neurological disorders who were given spinal anaesthesia). The mean cerebrospinal fluid adenosine deaminase activity was respectively

6.43 ± 1.93; 1.89 ± 0.91; 0.90 + 0.45 and 0.64 ± 0.57 IU/L in tuberculous meningitis, pyogenic meningitis, aseptic meningitis and controls. The adenosine deaminase activity in tuberculous meningitis cases was significantly higher [16]. The sensitivity and specificity of this test for diagnosis of tuberculous meningitis was 100% and 97.87% respectively with ADA value of more than 3.30 IU/L. Thus, adenosine deaminase activity in CSF, a relatively inexpensive and easy procedure, can be of great value in the diagnosis of tuberculous meningitis.

In the study conducted by Satya Vati Rana, Raj Kumar Singhal, Kartar Singh & Lata Kumar in Department of Paediatrics and Gastroenterology, Postgraduate Institute of Medical Education and Research, Chandigarh, Adenosine deaminase activity (ADA) was estimated in cerebrospinal fluid (CSF) of 30 patients of tuberculous meningitis (TBM) and 10 patients each of partially treated pyomeningitis (PTM), aseptic meningitis (AM) and pyogenic meningitis (PM) [18]. Mean ADA levels in CSF of TBM patients were higher (18.22 U/L) as compared to 6.28 U/L, 3.43 U/L and 7.98 U/L in PTM, AM and PM respectively. This difference of ADA values in CSF between TBM and other types of meningitis was statistically

significant ($p<0.01$) different. Sensitivity and specificity of ADA levels in CSF of children to diagnose tuberculous meningitis was 66.6% and 90% respectively at 10 U/L cut off of ADA levels in CSF. ADA levels in CSF could also differentiate PTM, AM and PM from TBM with a specificity of 90%, 100% and 80% respectively.

Gupta et al observed that adenosine deaminase levels in nontuberculous disease rarely exceeded the cut-off; set for tuberculous disease. They have further observed that ADA estimation is not only a fairly sensitive and specific test (more than 90 %), helpful in differentiating tubercular from non-tubercular etiology [14]; both in pulmonary and extra-pulmonary disease but is also simple, inexpensive and rapid. For this reason this test may help in early diagnosis, improve the prognosis and reduce spread of disease and sequelae.

Rajesh baheti, Purnima Laddha, RS Gehlot of Dr. SN Medical College, Jodhpur conducted a study in 38 patients of meningitis of varied etiology. Diagnosis was established by relevant investigations and patients were divided in different groups. CSF - ADA estimation was done by using spectrophotometer. The range of ADA was 6.2 to

21.8 (IU/L) in TBM with a mean of 12.23 (IU/L), while a range between 1.6 - 5.6 (IU/L) with a mean of 4.37 (IU/L) was noted with pyogenic meningitis. Similarly, ADA levels between 1.11 - 8.3 (IU/L) with 3.32 (IU/L) mean were observed with aseptic meningitis; whereas a range of 0.33 to 2.8 (IU/L) with a mean of 1.37 observed in control group [11]. CSF-ADA level 6.5 IU/L as a cut-off value exhibited 95.83% sensitivity and 92.85% specificity in differentiating tuberculous from non-tuberculous meningitis.

AIMS AND OBJECTIVES

1. To estimate the level of CSF Adenosine Deaminase in different types of meningitis &
2. To assess its usefulness in differentiating the various types (bacterial, viral and tuberculous) of meningitis.

MATERIALS AND METHODS

The study was conducted at the medical wards of Govt Rajaji Hospital, Madurai.

Design of Study : Prospective Analytical study

Period of Study : April 2012 to September 2012

Ethical committee approval:

Institutional Ethical Committee (IEC) of Madurai Medical College and Govt. Rajaji Hospital approved this research.

Collaborating departments:

General medicine, Neurology, Biochemistry, Microbiology.

Study Population:

Patients presenting with features suggestive of meningitis were the study subjects.

The diagnosis of meningitis was made on the basis of clinical symptoms and signs like headache, fever, nausea, vomiting, neck rigidity, presence of Kernig's and/or Brudzinski's sign, altered sensorium, any focal neurological deficit, cranial nerve palsies, seizures and/or signs of cerebral dysfunction ranging from

confusion, delirium, declining level of sensorium from lethargy to coma.

50 Consecutive medical ward in-patients of GRH who had meningitis were prospectively enrolled and included in the study after informed consent.

Inclusion criteria:

Age > 12 years.

Patients with features of meningitis.

Exclusion criteria:

Age <12 years.

Patients in whom lumbar puncture was contraindicated like

- Those with coagulopathy (Partial Thromboplastin Time > 50, international normalised ratio> 1.4, platelet count <1,00,000/mm^3)
- Those with local skin reactions and known spinal cord tumours.

Diagnostic classification of the subjects:

The different types of meningitis patients were separated on the basis of CSF cytochemistry and clinical features.

Tuberculous meningitis was confirmed if CSF culture yielded M. tuberculosis or a positive Ziehl - Neelson stain. Probable disease was diagnosed in the presence of a lymphocytic pleocytosis in the CSF with high protein content and low glucose content, negative bacterial and fungal cultures.

Acute bacterial meningitis was diagnosed in patients with CSF neutrophilia, high protein content, low glucose content and a positive gram stain and bacterial culture.

Viral meningitis was diagnosed if there was predominantly lymphocytic pleocytosis in the CSF with a normal or mildly raised protein content, a normal glucose content and negative bacterial, fungal and mycobacterial cultures.

Clinical and Laboratory Data:

History, clinical examination and laboratory investigation were carried out in all patients and details recorded in a predesigned proforma. Important clinical details regarding duration of fever, signs of meningeal irritation, focal neurological deficits and cranial nerve palsies were elicited. Laboratory examination included CSF analysis (appearance, cell counts, biochemistry, Gram, AFB & India

ink stain), blood counts, blood culture & sensitivity, Mantoux test, HIV test. Based on clinical and laboratory data, patient's type of meningitis was confirmed and treatment started accordingly.

Collection of CSF sample:

This was done by lumbar puncture. Colour and cobweb formation was noted. Total and differential cell count was estimated. Biochemical analysis of protein, sugar, chloride and globulin was done. Microbiological workup of the sample was done to find out the etiological organism with the help of Gram's, Ziehl-Neelson and Indian ink stains. About 2 ml of CSF was used to find out the ADA level.

Estimation of CSF ADA:

CSF Adenosine deaminase level was measured at 37°C according to the method of Guisti and Galanti based on the Berthlot reaction that is the formation of coloured indophenol complex from ammonia liberated from adenosine and quantified spectrophotometrically [5]. One unit of ADA is defined as the amount of enzyme required to release 1 mmol of ammonia/min from

adenosine at standard assay conditions. Results were expressed as units per litre per minute (U/L/min).

Assessment of outcome:

The primary outcome studied was the CSF ADA level in various types of meningitis and comparison of the level of elevation in tuberculous with other types of meningitis.

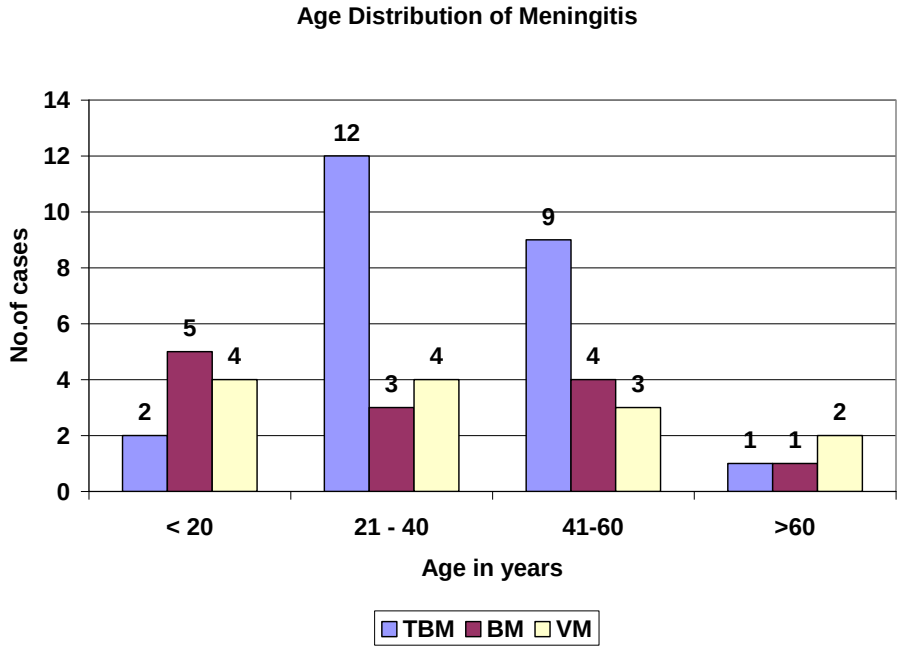
Statistical Analysis:

Descriptive statistical analysis was carried out in the study. Sigma Stat statistical software was used in the study. Microsoft word and Excel were used to generate graphs, tables etc. The CSF ADA mean values were calculated for the different meningitis category patients. One-way Anova & Chi square tests were used to determine statistical significance. Sensitivity, specificity, PPV, and NPV were calculated to know the diagnostic performance of ADA levels in relation to type of meningitis.

OBSERVATIONS AND RESULTS

Table 1- Age Distribution of Meningitis

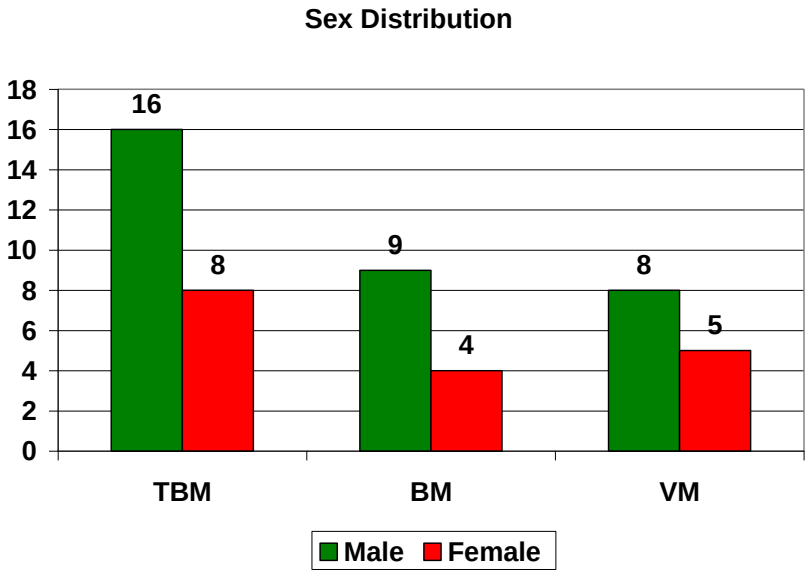
Age in years	No. of cases	TBM	BM	VM
<20	11	2	5	4
21-40	19	12	3	4
41-60	16	9	4	3
>60	4	1	1	2
Total	50	24	13	13



Tuberculous meningitis occurred more in the age group of 21 – 40 years. Bacterial meningitis was seen mainly in patients < 20 years of age. Viral meningitis was seen in all age groups

Table 2- Sex Distribution of Meningitis

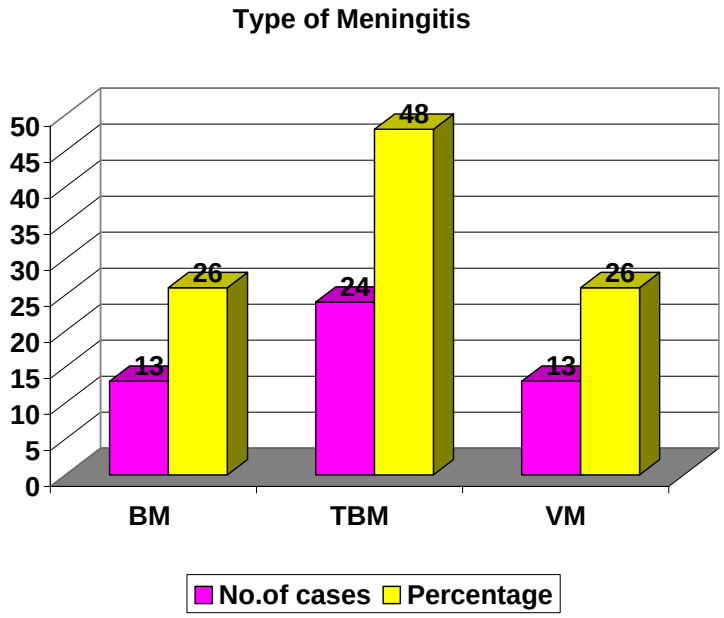
Sex	No. of cases	TBM	BM	VM
Male	33	16	9	8
Female	17	8	4	5
Total	50	24	13	13



Out of 50 cases, 33 were males. All three types of meningitis (tuberculous, bacterial and viral meningitis) were more common in males.

Table 3- Distribution of types of Meningitis

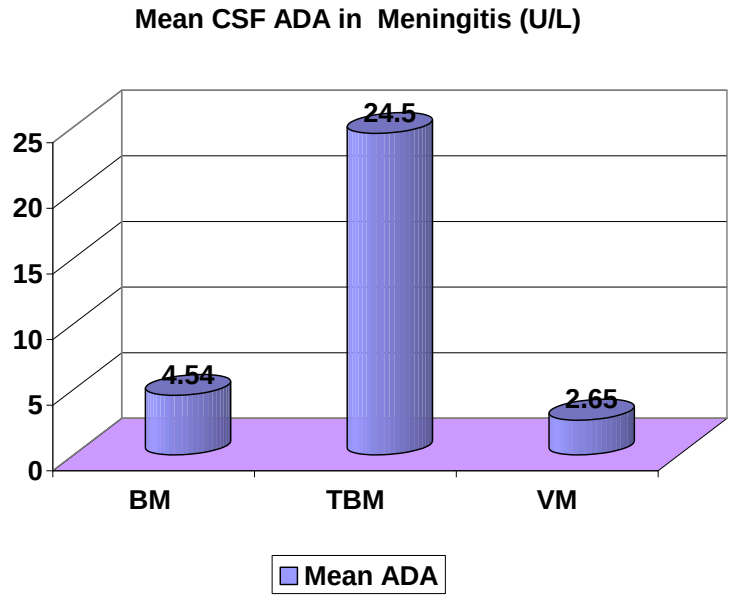
Type of Meningitis	No. of cases	% of cases
BM	13	26
TBM	24	48
VM	13	26
Total	50	100



Of the 50 cases, 24 had tuberculous meningitis. Viral and bacterial meningitis constituted 13 cases each. The percentages of tuberculous, bacterial and viral meningitis were 48%, 26% and 26% respectively.

Table 4- Mean CSF ADA level in various types of Meningitis

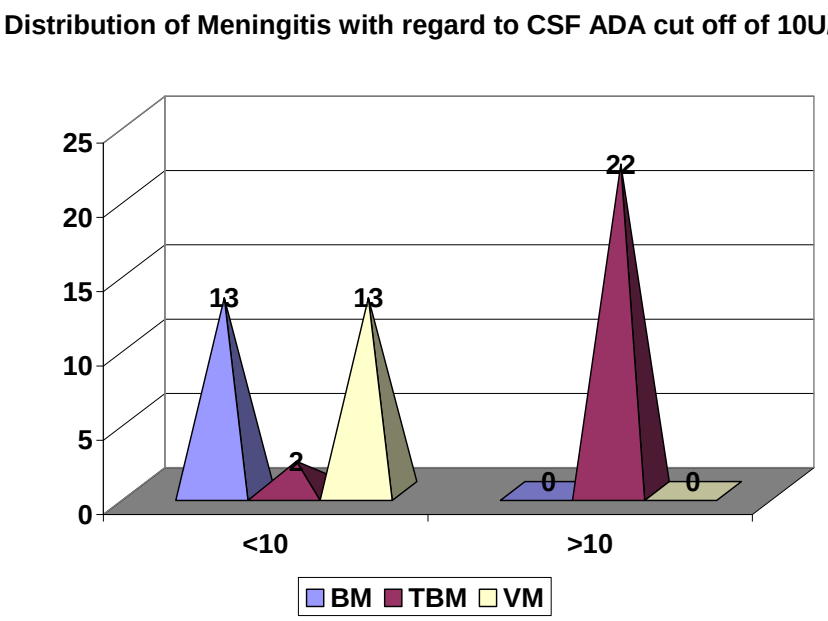
Type of Meningitis	Mean CSF ADA Level (U/L)
BM	4.54
TBM	24.5
VM	2.65



CSF ADA level was highest in tuberculous meningitis, the mean value being 24.5 U/L. The mean value of ADA in bacterial meningitis was 4.54 U/L and viral meningitis patients had lowest mean ADA value of 2.65 U/L.

Table 5- Distribution of Meningitis with respect to CSF ADA cut off level-10 U/L

CSF ADA Level (U/L)	No. of cases	BM	TBM	VM
<10	28	13	2	13
>10	22	-	22	-
Total	50	13	24	13



Of the 50 patients, 28 had ADA <10 U/L. Out of this, 26 cases belonged to the viral and bacterial meningitis groups and the rest 2 had tuberculous meningitis. About 22 patients had CSF ADA >10 U/L and all of them belonged to tuberculous meningitis group.

Table 6- Diagnostic Performance of CSF ADA (at 10 U/L cut off) in relation to the type of Meningitis

	Test (CSF ADA)		
Disease	>10	<10	Total
TBM	22 (a)	2 (c)	24 (a+c)
NonTBM (BM&VM)	0 (b)	26 (d)	26 (b+d)
Total	22 (a+b)	28 (c+d)	50

P value = < 0.001 (statistically significant)

Sensitivity = 22/22 * 100 = 100%

Specificity = 26/28 * 100 = 92.85%

Positive Predictive Value = 22/24 * 100 = 91.66%

Negative Predictive Value = 26/26 * 100 = 100%.

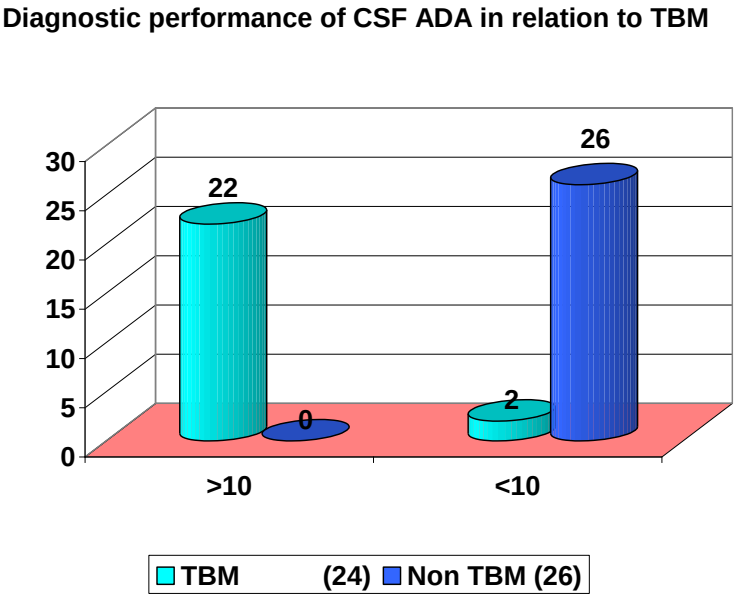
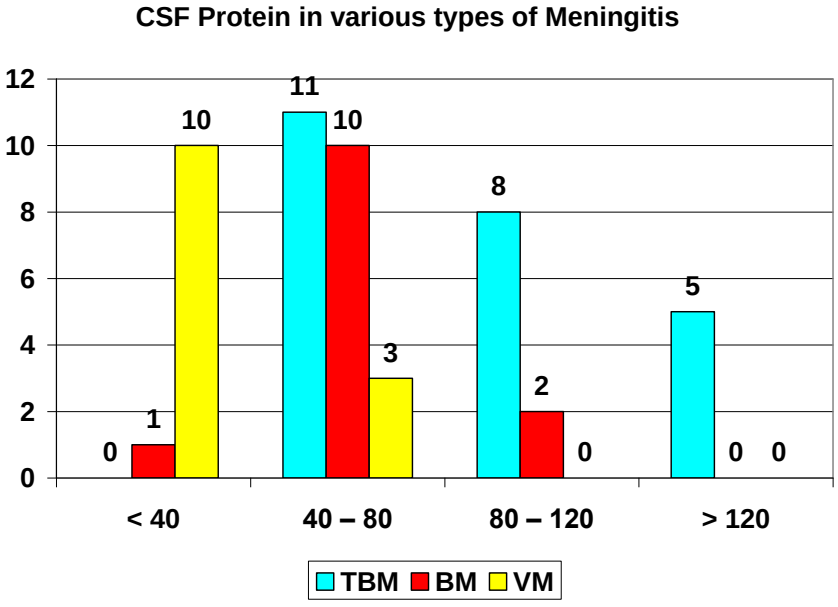


Table 7- CSF Protein level according to the type of Meningitis

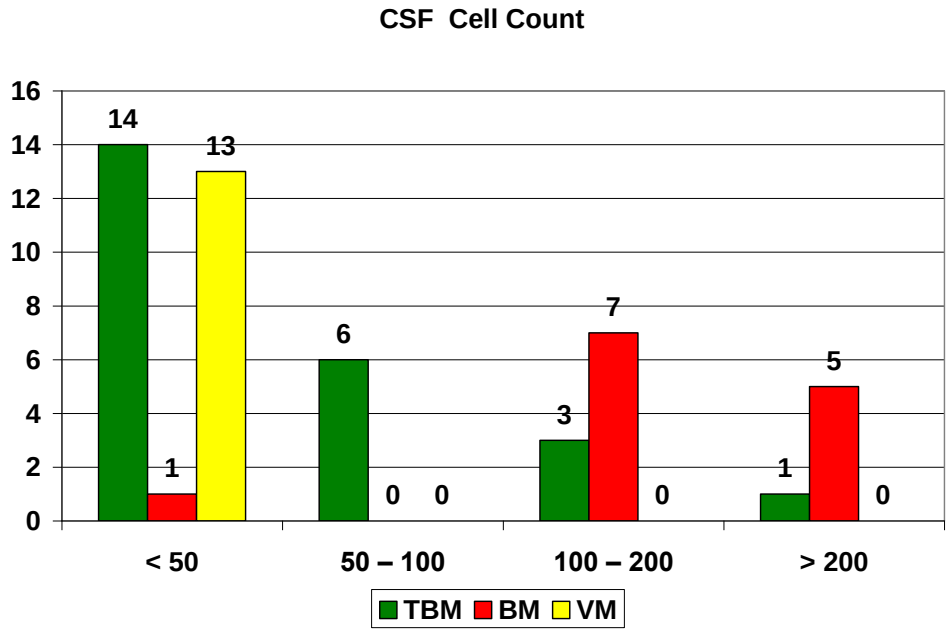
CSF Protein (mg/dL)	No. of cases	TBM	BM	VM
<40	11	0	1	10
40 - 80	24	11	10	3
80 - 120	10	8	2	0
>120	5	5	0	0
Total	50	24	13	13



Of the TBM patients, majority had CSF protein level more than 80 mg/dL. Most of the viral meningitis patients had protein level less than 40 mg/dL. Among the bacterial meningitis patients, protein level was found to be between 40 – 80 mg/dL predominantly.

Table 8-CSF cell count analysis according to the type of meningitis

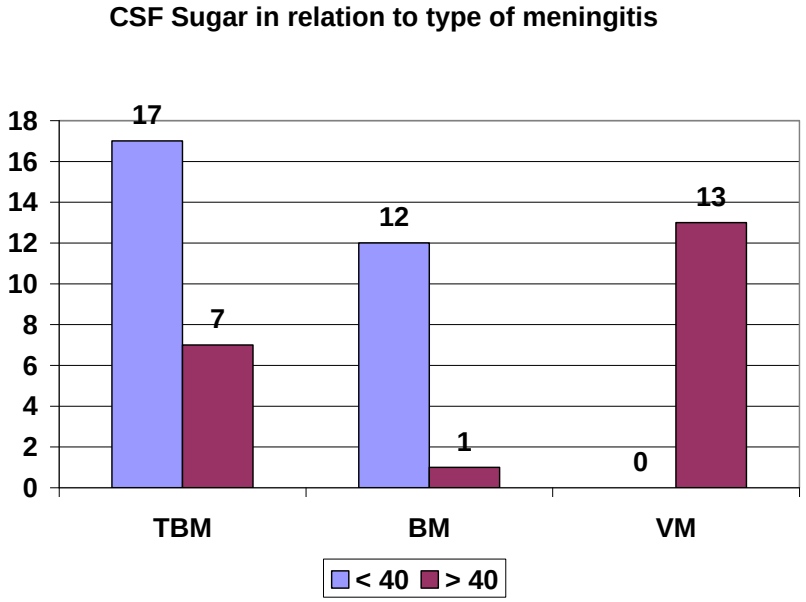
CSF cell count (cells/µL)	No. of cases	TBM	BM	VM
<50	28	14	1	13
50 -100	6	6	0	0
100 – 200	10	3	7	0
>200	6	1	5	0
Total	50	24	13	13



In TBM group, 14 patients had cell count less than 50 cells/µL and 10 patients had cell count more than 50 cells/µL. Most of the bacterial meningitis patients had cell count more than 100 cells/µL. All the viral meningitis patients had cell count less than 50 cells/µL.

Table 9- CSF sugar level according to the type of Meningitis

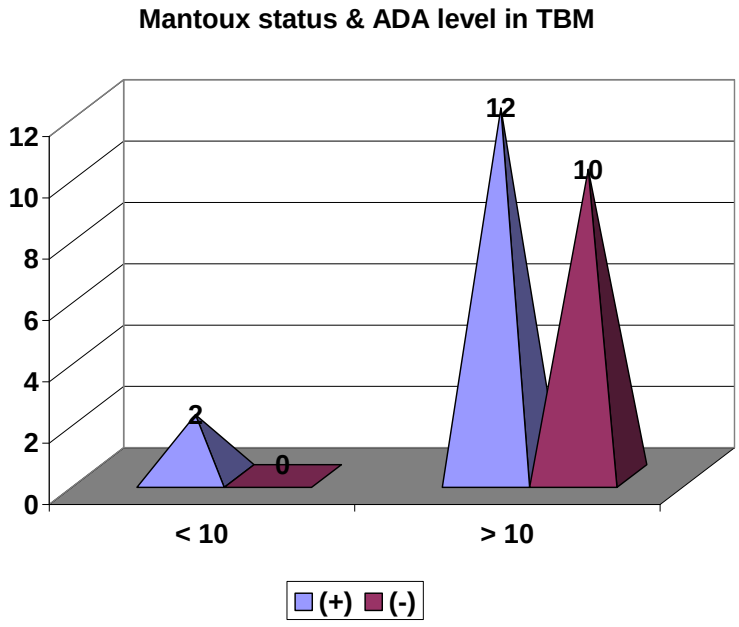
CSF Sugar (mg/dl)	No. of cases	TBM	BM	VM
<40	29	17	12	0
>40	21	7	1	13
Total	50	24	13	13



In tuberculous and bacterial meningitis group, majority had low sugar (<40 mg/dL). All the viral meningitis patients had normal CSF sugar.

Table 10- Mantoux status with regard to CSFADA level in Tuberculous Meningitis

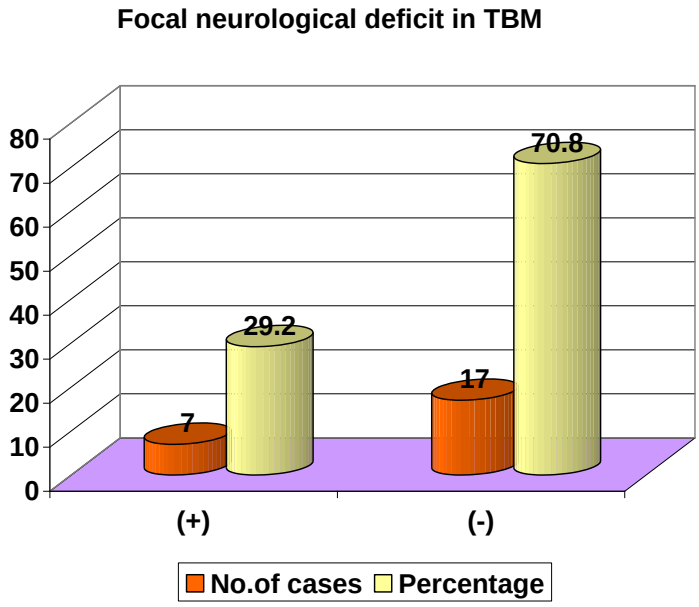
Mantoux/ CSF ADA	<10 U/L	>10 U/L
Positive	2	12
Negative	0	10
Total	2	22



Of the 24 TBM patients, 14 had mantoux positivity and 10 were mantoux negative. Of the 14 mantoux positive TBM patients, 12 had ADA more than the cut off level i.e. more than 10 U/L.

Table 11- Presence of Focal Neurological Deficit in TBM

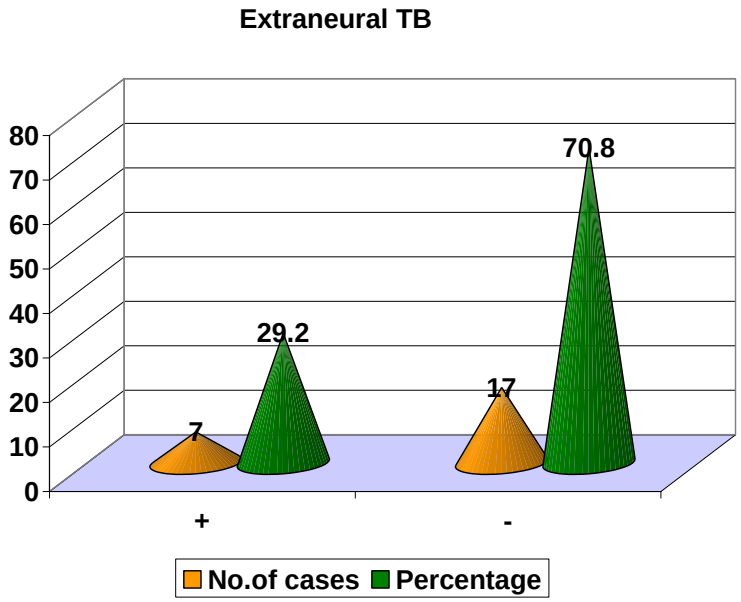
Focal Neurological Deficit	No. of cases	% of cases
Present	7	29.2
Absent	17	70.8
Total	24	100



Of the 24 TBM patients, 7 had developed focal neurological deficits and cranial nerve palsies. Of these 7 patients, one had Right hemiparesis, one had left monoparesis, and one had paraparesis. 2 patients had developed 3rd cranial nerve palsy and another 2 had developed 6th cranial nerve palsy.

Table 12- Extraneural TB (Pulmonary TB) in patients with TBM

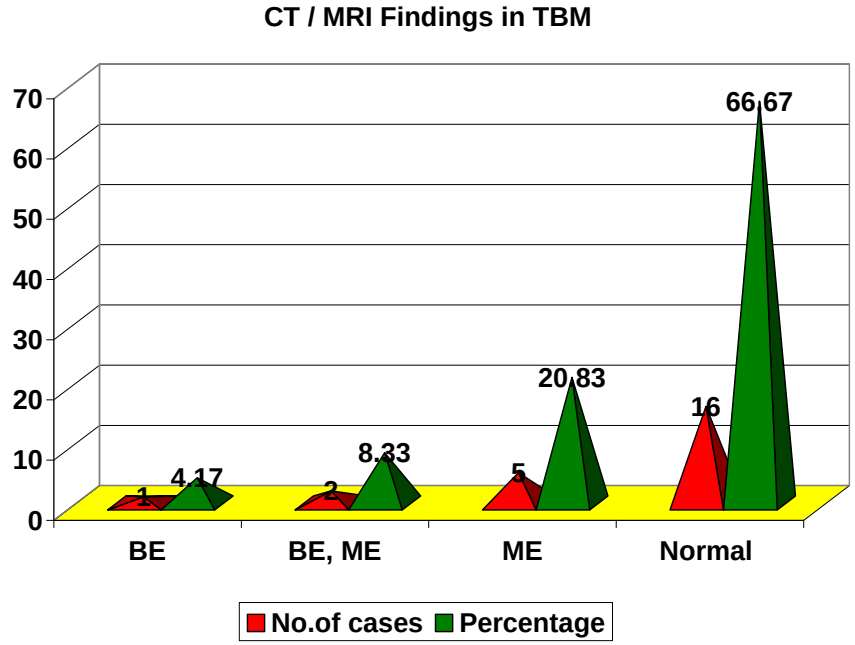
Extraneural TB	No. of cases	% of cases
Present	7	29.2
Absent	17	70.8
Total	24	100



Pulmonary TB was associated in 7 of the 24 TBM patients.

Table 13- CT/MRI Findings in patients with TBM

CT/MRI Findings	No. of cases	% of cases
BE	1	4.17
ME	5	20.83
BE & ME	2	8.33
Normal	16	66.67
Total	24	100



Of the 24 patients with TBM, 8 had positive findings in neuroimaging in the form of basal exudates (4.17%), meningeal enhancement (20.83%) and both meningeal enhancement and basal exudates together in 8.33% patients.

DISCUSSION

There is considerable urgency in establishing the correct diagnosis in patients with meningitis since specific therapy is most effective when instituted early in the course of illness. While waiting for cultures to confirm the diagnosis, irreversible brain damage can occur. Clinical features, CSF cytology, biochemistry and microbiology results are many a time inconclusive with regard to confirmation of diagnosis. So in suspected meningitis patients, specific treatment for tuberculous or non-tuberculous (bacterial or viral) etiology is usually begun on the basis of presumptive clinical diagnosis.

Moreover in the diagnostic evaluation of meningitis, sophisticated methods are necessary which are not routinely available in government institutions in developing countries like ours. In this situation, CSF Adenosine deaminase (ADA) estimation which is simple, cost effective and rapid, helps in differentiating the etiology of meningitis when other findings are ambiguous. Hence this simple test aids in prompt and appropriate treatment of this medical emergency and helps in saving many lives.

A total of 50 clinically suspected cases of meningitis admitted in GRH, were studied. 50 cases comprised of tubercular meningitis-24, bacterial meningitis-13 and viral meningitis-13 cases. CSF ADA activity was estimated in these patients.

Adenosine deaminase level was clearly higher in patients with tuberculous meningitis in our study, consistent with various earlier studies [7, 8, 15, 17, 21, and 22]. By using 10 U/L as the cut off value, the test had sensitivity of 100% and specificity of 92.85% in identifying cases with tuberculous meningitis. The positive predictive value and the negative predictive value were 91.66% and 100% respectively. The higher level of ADA in tuberculous meningitis was statistically significant ($p<0.001$).

In comparison to our study, Gupta et al in their study established that CSF ADA level of 10 U/ L as a cut off value exhibited 94.73 % sensitivity and 90.47% specificity in differentiating tuberculous from non-tuberculous meningitis. Various other studies used different cut off level for ADA, as 3.3 U/L in the study by Rajendra Prasad et al with sensitivity of 100% and specificity of 97.87%, 6.5 U/L in the study done by Rajesh

Baheti with sensitivity of 95.83 % and specificity of 92.85% and 15.5 U/L in the study by Verajit Chotmongkol in Thailand with sensitivity and specificity of 75% and 93% respectively.

The mean ADA level in CSF in cases with tuberculous meningitis was 24.5 U/L. It is considerably higher than that reported by other workers (11.7- 15.7 U/L). The range of CSF ADA level in TBM in our study was 6.0 U/L to 84.3 U/L.

The mean ADA level in CSF in bacterial meningitis and viral meningitis was 4.54 U/L and 2.65 U/L respectively. This is in comparison to the mean ADA level of bacterial meningitis (3.80±1.92 IU/L) and viral meningitis (1.85±1.43 IU/L) in the study done in Sri Siddhartha Medical College, Tumkur.

Among the nontuberculous etiology, thus ADA level was helpful in differentiating between viral and bacterial meningitis.

Age and Sex distribution of meningitis:

Most of the patients belonged to the 21-40 years age category followed by 41-60 years age group. Majority of the tuberculous meningitis patients belonged to the 21-40 years age group. Bacterial

meningitis patients were younger (<20 years). Viral meningitis occurred in all age groups.

There was a male predominance of patients in our study, male to female ratio of 1.9:1

CSF Biochemistry Characteristics:

Higher CSF protein levels were found in tuberculous and bacterial meningitis patients and 5 patients with TBM had CSF protein more than 120mg/dL and one had protein level of 2600mg/dL.

Higher CSF cell counts were found mainly in patients with bacterial meningitis and 5 of them had cell count more than 200 cells/μL.

Lower CSF sugar levels were found in both tuberculous and bacterial meningitis patients.

Complications and associated characteristics of TBM:

Focal neurological deficits and cranial nerve palsies were found in 7 TBM patients. Mantoux positivity in tuberculous meningitis was found in 14 cases out of 24 cases. Extraneural TB was found in 7 TBM patients in the form of pulmonary TB. In TBM

patients, positive CT/MRI findings were observed in 8 of the 24 patients.

2 patients in our study were HIV positive and they had developed tuberculous meningitis. HIV positivity did not affect the ADA level in these patients.

In case of bacterial meningitis, gram stain positivity was found in 9 of the 13 cases.

These were the other observations in our study apart from the CSF ADA characteristics.

CONCLUSION

In our study, 50 patients with meningitis admitted in Government Rajaji Hospital from April 2012 to September 2012 were evaluated.

Meningitis predominantly affected people in the age group of 20-40 years in our study with a male: female ratio of 1.9:1.

Cases of tuberculous meningitis constituted 48% of the study group and bacterial and viral meningitis were 26% each.

CSF protein values were higher and sugar values lower in patients with tuberculous and bacterial meningitis. CSF cell counts were higher in patients with bacterial meningitis.

The conclusions that can be drawn from our study, regarding CSF Adenosine Deaminase (ADA) estimation are as follows:

- CSF ADA is a simple, time saving, inexpensive indirect test that helps in identifying the type of meningitis, differentiating tuberculous from nontuberculous etiology.
- Further among the nontuberculous group of meningitis, ADA values are lowest in viral meningitis and thus it can aid in distinguishing bacterial from viral etiology.

- CSF ADA estimation can be done at routine labs spectrophotometrically and assayed according to the method of Guisti.
- CSF ADA cut-off of 10U/L considered in our study has 100% sensitivity and 92.85% specificity in differentiating tuberculous from nontuberculous etiology as early as possible, facilitating immediate management of these patients preventing morbidity and mortality due to delay in management.

However, as the study was limited to a small population due to financial and laboratory constraints, analysis of a larger group would definitely give an insight into the further finer relationship between CSF ADA and clinical severity and outcome of meningitis patients.

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CASE PROFORMA

NAME :

AGE :

SEX :

CLINICAL PROFILE:

Fever Duration

Headache

Photophobia

Vomiting

Altered Sensorium

Signs of Meningeal Irritation

Neck Stiffness

Kernig’s Sign

Brudzinski’s Sign

Focal Neurological Deficits/ Cranial Nerve Palsies

Features of Extraneural TB

Past H/O of Pulmonary TB/ ATT Intake

Contact H/O For TB

LAB:	CSF:	BLOOD
	Colour	Hb
	Presence of Cobweb	TC
	Protein	DC
	Sugar	ESR
	Chloride	VCTC
	Globulin	Culture & Sensitivity
	Cells, TC, DC	Mantoux test
	Gram Stain, AFB Stain	
	Indian ink Stain	
	Culture & Sensitivity	
	ADA	

IMAGING:

- CHEST X RAY
- CECT/MRI
- Meningeal enhancement
- Basal exudates
- Tuberculoma

TYPE OF MENINGITIS:

MASTER CHART

S. No	Name	Age (Yrs)	Sex	Duration of Illness	Signs of Meningeal Irritation			Focal Neurological Deficit/Cranial Nerve Palsies	Extraneural Tuberculosis	CSF					Mantoux	CT/MRI	VCTC	CSF Staining			Type of Meningitis
					Neck Stifness	Kernig's	Brudzinski			Cell Count (Cells/µL)	Protein (mgs/dl)	Sugar (mgs/dl)	Globulin	ADA(U/L)				Gram	AFB	Indian Ink	
1	Gunasekaran	35	M	7 Days	+	+	+	+	+	98	120	28	+	14.32	+	ME	NR	-	-	-	TBM
2	Ulaganathan	50	M	14 Days	+	+	+	-	-	10	100	32	+	20.1	-	Normal	NR	-	-	-	TBM
3	Govindaraj	56	M	21 Days	+	+	-	+	+	24	76	34	_	28.4	+	Normal	NR	-	-	-	TBM
4	Vellaimmal	35	F	6 Months	+	+	-	-	-	110	250	19	+	21.3	-	Normal	NR	-	-	-	TBM
5	Najima	42	F	24 Days	+	+	+	+	+	40	2600	16	+	25	-	ME	NR	-	-	-	TBM
6	Murugesan	30	M	15 Days	+	+	-	+	+	40	110	40	+	25.3	-	Normal	NR	-	-	-	TBM
7	Selvam	15	M	5 Days	-	-	-	-	-	11	60	52	-	2.03	-	Normal	NR	-	-	-	VM
8	Selvakumar	26	M	14 Days	+	-	-	-	-	42	100	76	+	24.8	-	Normal	NR	-	-	-	TBM
9	Chellapandi	13	M	4 Days	+	+	+	-	-	800	96	20	+	2.5	-	Normal	NR	+	-	-	BM
10	Kanagavel	56	M	10 Days	+	-	-	-	-	60	66	31	+	15.8	+	Normal	NR	-	-	-	TBM
11	Subbaiah	74	M	5 Days	+	-	-	-	-	5	28	96	-	3	-	Normal	NR	-	-	-	VM
12	Sivapandi	23	M	15 Days	+	+	+	-	+	28	120	36	-	13	-	ME	NR	-	-	-	TBM
13	Manikandan	48	M	3 Days	-	-	-	-	-	4	39	68	-	5.14	-	Normal	NR	-	-	-	VM
14	Abinesh	13	M	16 Days	+	+	+	-	-	38	104	55	+	10.28	-	ME	NR	-	-	-	TBM

15	Ramar	17	M	4 Days	+	+	+	-	-	210	70	35	-	6.9	-	Normal	NR	+	-	-	BM
16	Kalimuthu	70	M	21 Days	+	+	+	+	-	1650	75	24	+	60.25	-	ME	NR	-	-	-	TBM
17	Karanthamalai	63	M	5 Days	+	+	+	-	-	5	22	80	-	3.7	-	Normal	NR	-	-	-	VM
18	Sandhya	14	F	2 Days	+	-	-	-	-	6	35	90	-	1.82	-	Normal	NR	-	-	-	VM
19	Sadham hussain	19	M	20 Days	+	+	+	-	-	110	70	57	+	6	+	Normal	NR	-	-	-	TBM
20	shanthi	55	F	20 Days	+	+	+	-	-	200	65	43	+	21.3	+	Normal	NR	-	-	-	TBM
21	Veeraiyah	36	M	4 Days	+	+	+	-	-	18	30	56	-	3.2	-	Normal	NR	-	-	-	VM
22	Palkis	19	F	5 Days	-	-	-	-	-	15	23	58	-	3.12	-	Normal	NR	-	-	-	VM
23	Abuthahir	45	M	2 Days	+	+	+	-	-	150	97	30	+	5.4	-	ME	NR	+	-	-	BM
24	Havamma	69	F	3 Days	+	+	+	-	-	158	51	29	-	5	-	ME	NR	+	-	-	BM
25	Subramniam	60	M	2 Days	+	-	-	-	-	5	28	59	-	2.2	-	Normal	NR	-	-	-	VM
26	Tamilselvi	33	F	5 Days	+	+	+	-	-	200	60	40	-	5.5	-	ME	NR	-	-	-	BM
27	RajaMani	56	F	10 Days	+	+	+	-	-	70	90	40	+	84.3	+	BE	NR	-	-	-	TBM
28	Rajendra Kumar	27	M	3 Days	-	-	-	-	-	250	62	38	-	4.5	-	Normal	NR	+	-	-	BM
29	Meenakshi	50	F	30 Days	+	-	-	-	-	30	72	30	+	13.7	+	Normal	NR	-	-	-	TBM
30	Banumathi	53	F	6 Days	-	-	-	-	-	20	30	62	-	2.1	-	Normal	NR	-	-	-	BM
31	Laser	35	M	15 Days	+	+	+	-	-	30	70	50	+	16.8	-	Normal	R	-	-	-	TBM
32	Mohammed	34	M	14 Days	+	+	-	-	-	24	68	22	+	26.4	+	Normal	NR	-	-	-	TBM
33	Sathish	18	M	4 Days	+	-	-	-	-	16	30	52	-	1.96	-	Normal	NR	-	-	-	VM
34	Karpagam	40	F	20 Days	+	-	-	-	-	34	80	36	+	17.3	+	Normal	NR	-	-	-	TBM
35	Anitha	26	F	12 Days	+	+	+	+	+	100	140	30	+	7.2	+	BE, ME	NR	-	-	-	TBM
36	Karuppusamy	15	M	2 Days	+	+	+	-	-	240	60	20	-	6.4	-	Normal	NR	+	-	-	BM
37	Geetha	35	F	5 Days	-	-	-	-	-	20	36	66	-	3.4	-	Normal	NR	-	-	-	VM
38	Arumugam	42	M	4 Days	+	-	-	-	-	160	70	18	-	5.04	-	Normal	NR	+	-	-	BM
39	Rajamurugan	20	M	5 Days	-	-	-	-	-	120	54	38	-	4.38	-	Normal	NR	-	-	-	BM

40	Saravanan	36	M	17 Days	+	+	-	-	-	40	86	42	+	32.16	+	Normal	NR	-	-	-	TBM
41	Rajesh	24	M	23 Days	+	-	-	-	-	46	68	36	+	29.23	+	Normal	NR	-	-	-	TBM
42	Saroja	32	F	6 Days	+	+	+	-	-	20	40	62	-	1.54	-	Normal	NR	-	-	-	VM
43	Marimuthu	19	M	2 Days	+	+	-	-	-	146	56	22	-	3.92	-	ME	NR	+	-	-	BM
44	Ibrahim	47	M	30 Days	+	+	+	+	+	40	100	42	-	17.68	-	ME, BE	R	-	-	-	TBM
45	Ramasamy	60	M	7 Days	+	-	-	-	-	18	36	70	-	2.14	-	Normal	NR	-	-	-	VM
46	Manju	21	F	6 Days	+	-	-	-	-	250	46	30	-	4.32	-	Normal	NR	+	-	-	BM
47	Sakunthala	34	F	21 Days	+	+	+	-	-	80	58	40	+	36.42	+	Normal	NR	-	-	-	TBM
48	Manikkam	49	M	4 Days	+	+	+	-	-	180	54	20	-	3.1	-	Normal	NR	-	-	-	BM
49	Valarmathi	27	F	2 Days	+	-	-	-	-	16	40	57	-	1.23	-	Normal	NR	-	-	-	VM
50	Palaniappan	54	M	60 Days	+	-	-	-	-	70	62	32	-	21.46	+	Normal	NR	-	-	-	TBM

ABBREVIATIONS

+	-	Present
-	-	Absent
ADA	-	Adenosine Deaminase
AFB	-	Acid Fast Bacilli
BE	-	Basal Exudates
BM	-	Bacterial meningitis
CSF	-	Cerebrospinal fluid
CT	-	Computed Tomography
FND	-	Focal Neurological Deficit
HIV	-	Human Immunodeficiency Virus
LP	-	Lumbar Puncture
ME	-	Meningeal Enhancement
MRI	-	Magnetic Resonance Imaging
Mx	-	Mantoux
NR	-	Non Reactive
PCR	-	Polymerase chain reaction
R	-	Reactive
TBM	-	Tuberculous meningitis
VCTC	-	Voluntary Testing and Counselling Centre
VM	-	Viral meningitis

ETHICAL CLEARANCE APPROVAL

Ref. No. 5336 /E4/3/2012 **Govt. Rajaji Hospital, Madurai.20. Dated: .08.2012**

Institutional Review Board / Independent Ethics Committee.
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Sub: Establishment-Govt. Rajaji Hospital, aMadurai-20-
Ethics committee-Meeting Agenda-communicated-regarding.

The Ethics Committee meeting of the Govt. Rajaji Hospital, Madurai was held at 11.00 Am to 1.00Pm on 28.06.2012 at the Dean Chamber, Govt. Rajaji Hospital, Madurai. The following members of the committee have been attended the meeting.

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1. Dr.N.Vijayasankaran,M.ch(Uro.) 094-430-58793 0452-2584397	Sr.Consultant Urologist Madurai Kidney Centre, Sivagangai Road,Madurai	Chairman
2. Dr.P.K. Muthu Kumarasamy, M.D., 9843050911	Professor & H.O.D of Medical, Oncology(Retired)	Member Secretary
3. Dr.T.Meena,MD 094-437-74875	Professor of Physiology, Madurai Medical College	Member
4. Dr. S. Thamilarasi, M.D (Pharmacol)	Professor of pharmacology	
5.Dr.Moses K.Daniel MD(Gen.Medicine) 098-421-56066	Professor of Medicine Madurai Medical College	Member
6.Dr.M.Gobinath,MS(Gen.Surgery)	Professor of Surgery Madurai Medical College	Member
7.Dr.S. Dilshadh, MD(O&G) 9894053516	Professor of OP&Gyn Madurai Medical College	Member
8.Dr.S.Vadivel Murugan., M.D, 097-871-50040	Professor of Medicine Madurai Medical College	Member
9.Shri.M.Sridher,B.sc.B.L. 099-949-07400	Advocate, 2, Deputy collectors colony 4th street KK Nagar, Madurai-20.	Member
10.Shri.O.B.D.Bharat,B.sc., 094-437-14162	Businessman Plot No.588, K.K.Nagar,Madurai.20.	Member
11.Shri. S.sivakumar,M.A(Social) Mphil 093-444-84990	Sociologist, Plot No.51 F.F, K.K Nagar, Madurai.	Member

Following Projects were approved by the committee

Sl. No	Name of P.G.	Course	Name of the Project	Remarks
1.	Dr. Sandhya	M.D Gen med	CSF levels of ADA in meningitis.	Approved

Please note that the investigator should adhere the following: She/He should get a detailed informed consent from the patients/participants and maintain Confidentially.

1. She/He should carry out the work without detrimental to regular activities as well as without extra expenditure to the institution to Government.
2. She/He should inform the institution Ethical Committee in case of any change of study procedure site and investigation or guide.
3. She/He should not deviate for the area of the work for which applied for Ethical clearance.

She/He should inform the IEC immediately, in case of any adverse events pr Serious adverse reactions.

4. She/he should abide to the rules and regulations of the institution.
5. She/He should complete the work within the specific period and apply for if any Extension of time is required She should apply for permission again and do the work.
6. She/He should submit the summary of the work to the Ethical Committee on Completion of the work.
7. She/He should not claim any funds from the institution while doing the word or on completion.
8. She/He should understand that the members of IEC have the right to monitor the work with prior intimation.

DEAN 12.8.12

54/58

To
All the above members and Head of the Departments concerned.
All the Applicants.

ANTI-PLAGIARISM CERTIFICATE